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Your reference PH.36402/MAP.

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Notes

Please type, or write in dark ink using CAPITAL letters. A prescribed fee is payable for a request for grant of a patent. For details, please contact the Patent Office (telephone 071–829 6910).

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The Patent Office

Request for grant of a Patent

Form 1/77

Patents Act 1977

❶ Title of invention

1 Please give the title of the invention: PEPTIDE PROCESS

❷ Applicant's details

☐ **First or only applicant**

2a If you are applying as a corporate body please give:

Corporate name: IMPERIAL CHEMICAL INDUSTRIES PLC

Country *(and State of incorporation, if appropriate)*: United Kingdom

2b If you are applying as an individual or one of a partnership please give in full:

Surname

Forenames

2c **In all cases**, please give the following details:

Address: Imperial Chemical House,
Millbank,
London.

UK postcode *(if applicable)*: SW1P 3JF

Country: United Kingdom

ADP number *(if known)*: 935003

2d, 2e and 2f: If there are further applicants please provide details on a separate sheet of paper.

☐ **Second applicant** ***(if any)***

2d If you are applying as a corporate body please give:

Corporate name

Country *(and State of incorporation, if appropriate)*

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Surname

Forenames

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⊕ *An address for service in the United Kingdom must be supplied*

Please mark correct box

⊕ Address for service details

3a Have you appointed an agent to deal with your application?

Yes [X] No [ ] ➡ *go to 3b*

⬇ *please give details below*

Agent's name REGINALD PETER SLATCHER

Agent's address Legal Department: Patents
Imperial Chemical Industries PLC
P O Box 6, Bessemer Road
Welwyn Garden City
Hertfordshire

Postcode AL7 1HD

Agent's ADP number 01320837001

3b: If you have appointed an agent, all correspondence concerning your application will be sent to the agent's United Kingdom address.

3b If you have not appointed an agent please give a name and address in the United Kingdom to which all correspondence will be sent:

Name

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Daytime telephone number *(if available)*

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❹ Reference number

4 Agent's or applicant's reference number *(if applicable)* PH. 36402/AA/P

❺ Claiming an earlier application date

5 Are you claiming that this application be treated as having been filed on the date of filing of an earlier application?

Please mark correct box

Yes ☐ No ☒ ➡ *go to 6*

⬇

please give details below

❑ number of earlier application or patent number

❑ filing date

(day month year)

❑ and the Section of the Patents Act 1977 under which you are claiming:

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15(4) (Divisional) ☐ 8(3) ☐ 12(6) ☐ 37(4) ☐

❻ Declaration of priority

❻ If you are declaring priority from a PCT Application please enter 'PCT' as the country and enter the country code (for example, GB) as part of the application number.

Please give the date in all number format, for example, 31/05/90 for 31 May 1990.

6 If you are declaring priority from previous application(s), please give:

Country of filing	Priority application number *(if known)*	Filing date *(day, month, year)*

❼ *The answer must be 'No' if:*
- *any applicant is not an inventor*
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❼ Inventorship

7 Are you (the applicant or applicants) the sole inventor or the joint inventors?

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Yes [ ] No [X] ➡ ***A Statement of Inventorship on Patents Form 7/77 will need to be filed (see Rule 15).***

❽ *Please supply duplicates of claim(s), abstract, description and drawing(s).*

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8a Please fill in the number of sheets for each of the following types of document contained in this application.

Continuation sheets for this Patents Form 1/77			
Claim(s)		Description	6
Abstract		Drawing(s)	

8b Which of the following documents also accompanies the application?

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Translation(s) of Priority documents *(please state how many)*	
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PEPTIDE PROCESS

This invention relates to a process for making peptides and more particularly it relates to a solid phase peptide synthesis method for the preparation, inter alia, of the decapeptide goserelin.

The solid phase synthesis of peptides has been known for almost 30 years following the pioneering work of Merrifield first published in 1962. The general principle of this type of synthesis is as follows:-

(a) An N-protected amino acid (the protecting group is commonly t-butoxycarbonyl, abbreviated to Boc) is attached to a solid, non-soluble support (commonly a polystyrene resin) at its carboxylic end via a linking group (commonly a benzyl ester).

(b) The N-protecting group is removed by means which do not detatch the amino acid from the solid support, and a second N-protected amino acid is coupled to the one already attached (commonly by use of a carbodi-imide coupling agent).

(c) The sequence is repeated using as many N-protected amino acids as are required until the desired peptide has been formed, still attached at its carboxyl end to the solid support.

(d) The final N-protecting group is removed and the peptide is separated from the solid support by cleavage of the linking group (commonly by use of a strong acid).

The whole synthesis can be machine-aided and in some circumstances the peptide may be formed without manual intervention. The Boc protecting groups are removed by triflouroacetic acid and the peptide chain is removed from the solid support with a stronger acid such as hydrofluoric acid.

Since the introduction of this technique many modifications have been introduced, but the process is essentially as first proposed. Two major

innovations have been the use of a polyamide as the solid support and the use of a N-fluoren-9-ylmethoxycarbonyl (Fmoc) protecting group for the N^{α}-group of the amino acid. The Fmoc group is distinguished by being labile to base (commonly piperidine). For further detail reference is made, for example, to Atherton and Sheppard, "Solid phase peptide synthesis - a practical approach", IRL Press at Oxford University Press, 1989; Barany et al., "Solid-phase peptide synthesis: a silver anniversary report", Int. J. Peptide Protein Res., 1987, 30, 705-739 and Fields et al., ibid, 1990, 35, 161-214.

Throughout this specification standard abbreviations for amino acids, protecting groups, coupling agents and the like will be used. For the avoidance of doubt, as well as Boc and Fmoc defined above, the following are relevant standard abbreviations:-

Arg	arginine
Azgly	azaglycine (H_2N-NH-COOH)
D-Ser	D-serine
Glp	pyroglutamic acid
His	histidine
Leu	leucine
Pro	proline
Ser	serine
Trp	tryptophan
Tyr	tyrosine

DIPC	di-isopropylcarbodi-imide
HOBt	1-hydroxybenzotriazole
DMF	N,N-dimethylformamide
BrZ	2-bromobenzyloxycarbonyl
Bu^t	tert-butyl
Bzl	benzyl

Goserelin is an LHRH analogue used in the treatment of prostate cancer, breastcancer and certain gynaecological conditions. In the first-mentioned treatment it acts by inducing a chemical castration.

Its structure is:-

Glp-His-Trp-Ser-Tyr-D-Ser(Bu^t)-Leu-Arg-Pro-Azgly-NH_2

It will be seen that there are two features of this structure which are incompatible with traditional solid phase peptide synthetic routes. The first is the Azgly carboxy terminal amino acid; procedures for linking such a group to a solid support are not known. Free azaglycine has a terminal -NH-COOH group, which is an unstable carbamic acid.

The second is the t-butyl group attached to the D-serine moeity; in order to preserve this group traditional means for removing the completed peptide from the solid support cannot be used.

We have nov found a method of preparing goserelin and similar peptides by solid phase synthesis.

According to the invention there is provided a method for solid phase synthesis of a peptide containing a C-terminal aza-amino acid amide, which comprises
(i) assembling all the amino acids of the peptide except the C-terminal aza-amino acid by conventional solid phase synthesis;
(ii) cleaving the peptide from the support with hydrazine or a substituted hydrazine; and
(iii) reacting the hydrazide thus released with a cyanate ion.

The last two stages of this process form firstly a peptide with the carboxyl end of the formula:-

-CONH-NH-R

wherein R is hydrogen (in azgly) or such a group that H_2N-NR-COOH is an aza-analogue of an amino acid, and secondly a peptide with the carboxyl end of the formula:-

-CONH-NR-$CONH_2$

The cleavage of the peptide from the support may be carried out using hydrazine or a substituted hydrazine in solution in DMF, N-methylpyrrolidone or a similar solvent.

A suitable cyanate ion may be provided by an alkali metal cyanate, for example potassium cyanate. The reaction may be carried out in aqueous acidic conditions.

According to a further feature of the invention there is provided a method for solid phase synthesis of a peptide containing an amino acid which contains a t-butyloxy group in its sidechain which comprises the use of a linking group connecting the amino acid to the solid support which is labile under conditions which do not cleave an O-t-butyl group.

A suitable linking group is one which may be cleaved by the use of hydrazine which will not cleave the t-butyl ether.

The amino acids contained in such a peptide are the t-butyl ethers of, for example, serine, D-serine, threonine, tyrosine and hydroxyproline.

The invention is illustrated but not limited by the following example:-

Example

(a) Solid phase perparation of nonapeptide

The solid phase synthesis was carried out in automatic mode on an Applied Biosystems 430A Peptide Synthesizer using Boc-Pro-OBzl-polystyrene resin 1% cross-linked with divinylbenzene (Peninsula Laboratories, 1.25g, 0.38 meq/g though nominally 0.7 meq/g). The following protected amino acids were converted to benzotriazolyl esters by reaction with HOBt and DIPC in DMF immediately before use. The protected amino acids were coupled in the following sequence:-

Boc-Arg(HCl)-OH
Boc-Leu-OH
Fmoc-DSer(Bu^t)-OH
Fmoc-Tyr(BrZ)-OH
Fmoc-Ser-OH
Fmoc-Trp-OH
Fmoc-His(Fmoc)-OH
Pyr-OH

The sequence of operations for the first two stages (using Boc-protected- amino acids) was:-

removal of Boc with 45% triflouroacetic acid in methylene chloride
10% DIEA/DMF wash
coupling (2 equivalents of protected amino acid HOBt ester)
removal of Boc as above

The sequence of operations for the last six stages (using Fmoc-protected-
amino acids) was:-

removal of Fmoc with 20% piperidine/DMF
0.5 molar HOBt/DMF wash
coupling (1 equivalent of protected amino acid HOBt ester)

All coupling reactions except that using Boc-Arg(HCl)-OH were of 1 hour duration; the Boc-Arg(HCl)-OH one was of 2 hours duration. There was thus obtained the nonapeptide-resin (1.7g; 0.29 mmole peptide per g.) with the Tyr still protected by BrZ.

(b) Cleavage of peptide from resin

The peptide resin prepared above was treated with a 20-fold excess of anhydrous hydrazine in DMF (20ml) at laboratory temperature for 24 hours, and the mixture was filtered and evaporated to dryness. This procedure also removed the BrZ protecting group from the Tyr moeity.

The residue was purified by gel filtration on a column (LH 20 Sephadex) using a 20:1 v/v mixture of water and acetic acid as eluant. There was thus obtained

$$Pyr\text{-}His\text{-}Trp\text{-}Ser\text{-}Tyr\text{-}DSer(Bu^{t})\text{-}Leu\text{-}Arg(H^{+})\text{-}Pro\text{-}NH\text{-}NH_{2}$$

the structure of which was confirmed by amino acid analysis and FAB mass spectroscopy $(M+H)^{+}$ = 1226.

(c) Preparation of goserelin

A solution of potassium cyanate (11mg) in water (1.36ml) was added portionwise during 1 hour to a solution of the above hydrazide (118mg) in a 20:1 v/v mixture of water and acetic acid (10ml). The mixture was freeze-dried and the residue was purified by reverse-phase column chromatography (Dynamax 60Å, C_{18}, 1 inch diameter) using a gradient of 10% to 40% by volume of acetonitrile in water containing 0.1% trifluoroacetic acid. There was thus obtained goserelin (100mg, 25% yield overall), the structure of which was confirmed by FAB mass spectroscopy.